

Temperature Effect on the Colloidal Mastic Test

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The colloidal mastic test is widely used as one of the laboratory diagnostic aids in the management of central nervous system syphilis. The procedure is highly sensitive to slight changes in laboratory technique, and it has been thought that the temperature at which the test is performed may influence the results.

The sensitivity of the colloidal mastic test is affected by several factors, such as electrolyte concentration, type of electrolyte, and size of the colloidal particles. When these factors are adequately controlled, reasonably reproducible results are obtained. However, there are times when the sensitivity of the test varies from the usual or expected level, indicating that there are other factors concerned. Since temperature changes affect activities of colloids, and no reference could be found in the literature, this study was initiated to determine the effect of various temperatures on the sensitivity of the colloidal mastic test.

Materials and Methods

Three reagents were prepared in sufficient quantities for 270 tests: a 10-percent solution of gum mastic in absolute ethyl alcohol; a 1.25-percent sodium chloride solution; and a 5-percent solution of potassium carbonate in freshly distilled water. Cutting's (1) modification of the colloidal mastic test was used, and the sensitivity adjusted with optimally alkalinized salt solution. Dehydrated stable control serum (2)

was used as the constant source of specimen material in the test performance.

Sufficient dehydrated stable control serum was reconstituted to the proper volume for use as a control for colloidal mastic tests. The control was apportioned in stoppered test tubes in amounts sufficient for one day's testing and frozen until needed. For each day's tests, one tube was thawed and 100 ml. of a 1:4 dilution was made with optimally alkalinized 1.25-percent salt solution. Ten serial dilutions, using alkalinized 1.25-percent salt solution as the diluent, were made in 50-ml. accounts, as follows: 1:8, 1:16, up to 1:2,048. One milliliter of each dilution was placed in each of 27 tubes, making a series of 27 tests of 10 tubes each, with the control serially diluted from 1:4 in the first tube to 1:2,048 in the tenth tube. Nine sets of dilutions were placed in the 37° C. incubator, 9 sets left at room temperature, and 9 sets placed in the refrigerator. To permit the tubes and dilutions to reach the desired temperature, each set was allowed to stand at room, refrigerator, and incubator temperatures for 1 hour before adding the colloidal mastic. Temperature ranges during the testing period were: room, 5° to 26° C. (minimum, 5° to 15° C.; maximum, 21° to 26° C.); refrigerator, 4° to 6° C.; incubator, 37° ± 1° C.

Three mixes of precipitated mastic were made each day. Each mix was precipitated by adding 2 ml. of 10-percent mastic solution to 18 ml. of 95-percent ethyl alcohol and rapidly pouring the 1-percent mastic solution into 80 ml. of freshly distilled water, with moderate agitation. One mix and one lot of distilled water were allowed to stand at each of three temperatures (incubator, room, and refrigerator) for 5 hours prior to mixing.

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Three groups of dilutions of nine sets each were maintained at incubator, room, and refrigerator temperatures; the mastic solutions, which had been precipitated at the same three temperatures, were added to the dilutions; and the tests allowed to remain overnight at these temperatures. Thus, the effect of temperature on the sensitivity and reproducibility of the colloidal mastic test could be studied in relation to temperature at which dilutions were maintained prior to addition of colloidal mastic, temperature of the alcoholic mastic solution and water at the time of precipitation of the mastic, and temperature at which the tests were maintained overnight.

Results

Test results are shown in the accompanying table. The figure given for each dilution represents the arithmetic average obtained from the results of 10 tests performed in an identical manner on 10 consecutive workdays. The daily results did not vary more than $2 \pm$ from that shown as average for any given dilution.

Discussion

The table indicates that the overnight temperature has the greatest influence on the sensitivity of the test, and that the most consistent, and most sensitive, results were obtained when the tests were allowed to remain overnight in the 37° C. incubator, regardless of the temperature at which the mastic was precipitated or the temperature of the dilutions when the mastic was added. Test results were most uniform when the tubes remained overnight in the incubator. Some decrease in sensitivity was noted when the tubes stood overnight at room temperature, and sensitivity decreased further at refrigerator temperature.

The temperature extremes of 37° and 4° C. were selected for evaluation, since it is not uncommon for seasonal changes to produce fluctuations of this magnitude. These incubator and refrigerator temperatures are normally maintained in most laboratories, and therefore the temperature of choice could be standardized without complicating routine conditions.

Temperature combinations and results

Dilution temperature ¹	Colloidal mastic temperature ²	Overnight temperature	Results ³
Incubator (37° C. ± 1° C.).	Incubator	Incubator Room Refrigerator	5554321000 5553210000 5532110000
	Room	Incubator Room Refrigerator	5554321000 5543210000 5532100000
	Refrigerator	Incubator Room Refrigerator	5554321000 5543210000 5532100000
Room (5° to 26° C.).	Incubator	Incubator Room Refrigerator	5554321000 5543210000 5532100000
	Room	Incubator Room Refrigerator	5554321000 5542100000 5531000000
	Refrigerator	Incubator Room Refrigerator	5554321000 5543210000 5431000000
Refrigerator (4° to 6° C.).	Incubator	Incubator Room Refrigerator	5554321000 5542100000 5521000000
	Room	Incubator Room Refrigerator	5554321000 5542100000 5421000000
	Refrigerator	Incubator Room Refrigerator	5554321000 5543210000 5431000000

¹ Prior to addition of precipitated mastic.

² Alcoholic mastic solution and water at time of precipitation.

³ Average of 10 tests performed on each of 10 consecutive working days.

Conclusions

Use of a stable control serum in proper dilution as a constant source of specimen material indicates that a more uniform level of sensitivity and a higher degree of reproducibility will result with the colloidal mastic test if the tests are stored overnight in the 37° C. incubator.

REFERENCES

- (1) Eagle, H.: The laboratory diagnosis of syphilis. St. Louis, C. V. Mosby Co., 1937, pp. 258-261.
- (2) Cannefax, G. R.: A stable control serum for standardizing the sensitivity of tests used in the diagnosis and control of syphilis. J. Ven. Dis. Inform. 30: 169-174 (1949).